Control of Aedes breeding sites with a dual-action insecticidal coating is effective in reducing Dengue transmission: Cluster-randomised Controlled Trial Study

Statistical Analysis Plan

Ubication of study: Metropolitan Area of Cucuta, Colombia

Approval of the protocol: 11 March 2019

Statistical Analysis Plan

Study design and sample size

This is design study for a large cluster randomized trial on the impact of treating water containers with a protective paint. The sample size was calculated for detection of a 50% reduction in the House Index with > 99% power at 5% significance level. Given a baseline HI of 30%, an intra-cluster correlation coefficient (ICC) of 0.01 and a cluster size of 2,000 households it was found that a minimum of 12 clusters per study arm was needed [1]. The same result but with a lower power (68%) was found for a baseline DZC incidence of 3% and a 50% reduction in the intervention arm. A large number of households per cluster was needed as *Ae. aegypti* vectors are day-biters [2] and it was assumed that many household members stay within their neighbourhoods during the day (mainly housewives, small children and school children when the school is close to their house) where they are at risk to get infected. All estimations were done through The Shiny CRT Calculator which is a web-based app to determine sample size and power for cluster trials [3,4].

Data management and analysis

Descriptive analyse and double data entry was practiced (to minimize data entry errors) into a database using Microsoft Office Excel software by an assistant and supervised by the research team. The analysis was done using SPSS software version 28.0.1.1.

Socio-demographic data were entered in a database. We assessed the frequency of variables potentially associated with the outcomes of recent dengue virus infection (sex, age, educational level, peoples` mobility) and selfreported dengue fever in household members (5). Persons' Chi-square test ($\chi$2) was applied to determine the differences between population characteristics and DZC selfreported cases, and peoples` mobility across all settings. All statistical analyses were performed at a 0.05 significance level.

Entomological indices were analysed per cluster and overall to show the presence, distribution and abundance of *Ae. aegypti* and the breeding sites most productive for adult mosquitoes were identified [6].

House index (HI): Percentage of houses infested with larvae and/or pupae.
Container index (CI): Percentage of water-holding containers infested with larvae or pupae.
Breteau index (BI): Number of positive containers per 100 houses inspected.
Pupae per person (PPI): Number of pupae per person in each household. To estimate the pupal count for large container (more than 20 L) the methodology by Romero-Vivas [7] was used: According to water level, the number of pupae found was multiplied by a calibration factor.

For the before-after comparison and intervention vs. control arm the difference-in-differences (DiD) approach was applied, taking into account the clustering effect, and estimating the statistical significance through the P-value. Likewise, the reduction of dengue cases reported by SIVIGILA was estimated, according to the annual dengue incidence in intervention and control clusters [8]

Ethical considerations

The household questionnaire was only applied to adults who provided information related to the purpose of this study. No child or adolescent below the age of 18 was interviewed in this study. All people participating in the study were informed in local language through the study information sheet in a written and oral way. They were asked to sign the informed consent form. All participants were informed that their participation was voluntary and that their responses remained anonymous, therefore the study used numbers which replaced the names of individuals and codes which replaced the address of house. Before examining the domestic and peri-domestic water-holding containers, the field team requested permission to enter the house, did the inspection and collected entomological and sociodemographic data.
The study received approval from local health authorities in Cucuta and Norte de Santander and the study protocol was approved by the ethical committee of the Albert–Ludwigs-Universität (application number 141/19) in Freiburg, Germany

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